physiological pH range. In the presence of PI two opposing effects lead to a net charge that is similar to the charge found for $PI(4,5)P_2$ in the absence of PI. The enhanced negative charge in the membrane due to the presence of PI leads to an increased $PI(4,5)P_2$ protonation (reduced charge). This effect is opposed by PI/$PI(4,5)P_2$ hydrogen bond formation which results in increased deprotonation of the phosphomonoester groups. As a result, PI appears to have a minor effect on $PI(4,5)P_2$ ionization, however, fluorescence microscopy measurements of PC/PI/$PI(4,5)P_2$ GUVs show a pronounced effect on $PI(4,5)P_2$ morphology.

1120-Plat

Single Molecule Study of the Processive Ras/SOS Interaction

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Ras is a membrane-anchored small GTPase protein that plays an important role in regulating essential cellular functions such as proliferation, differentiation, and apoptosis. Its deregulation is a hallmark of many cancers and developmental defects. Son of Sevenless (SOS) is a guanine nucleotide exchange factor (GEF) enzyme that activates Ras by catalyzing the exchange of GDP to the GTP in Ras.

Previously, we have shown that in addition to the catalytic site, SOS has a catalytically inactive distal Ras-binding site, which allows SOS to localize and up-concentrate at Ras presenting membranes, dramatically increasing the Ras-GDP turnover rate. Together, the catalytic and allosteric sites form the catalytic core of SOS (SOScat). The existence of the extra binding site for Ras also raises the question of whether SOS is processive, capable of remaining surface bound via the distal binding site while catalyzing the nucleotide exchange of multiple Ras. In this study we employ various fluorescence-based methods such as Fluorescence Correlation Spectroscopy (FCS) and Total Internal Reflection Fluorescence (TIRF) microscopy on Ras functionalized supported lipid bilayers (SLBs) to demonstrate that SOScat is processive. Single molecule tracking of SOScat allows us to correlate the diffusion behavior between Ras and SOScat and further confirms the specific interaction.

By confining individual SOScat enzymes to micron-scale two-dimensional Ras-functionalized SLB ''reaction chambers'' we can simultaneously monitor enzymatic activity from hundreds of single SOScat, probing the variability in catalytic rate and processivity within the enzyme ensemble. Our data indicates that SOScat has a broad range of processivities ranging from a few up to a thousand turnovers.

1121-Plat

Partitioning of Cholesterol and Ganglioside GM1 in Phase Separated Lipid Bilayers Imaged by Secondary Ion Mass Spectrometry

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Secondary ion mass spectrometry has been used to image the distribution of cholesterol and ganglioside GM1 in model membranes composed of palmitoyl sphingomyelin (PSM) and dioleoylphosphatidylcholine (DOPC) best described as the canonical lipid-raft composition. Isotopic labeling or fluorination of each lipid bilayer component allowed the generation of molecule specific images using the NanoSIMS50L instrument at Stanford University. Simultaneous detection of six different ion species, including secondary electrons, were used to generate ratio images whose signal intensity values could be correlated to composition through the use of calibration curves from standard samples. Images of this system indicate the presence of three compositionally distinct phases corresponding to: 1) the interdomain region; 2) large domains ($d>3\mu m$); and, 3) small domains ($d=200nm\text{-}1\mu m$) localized within the large domains. Although semi-quantitative compositional analysis of these distinct phases suggests that both the small and large domains have similar cholesterol content, the large domains were also GM1 and DOPC-rich while the small domains were also PSM-rich. Since the interdomain region is primarily PSM-rich, this seems to suggest that the small domains do not correspond to kinetically trapped interdomain regions and that their more than doubled cholesterol content makes them instead a completely different phase. Furthermore, the average diameter ($d = 380nm \pm 230nm$) of these small domains suggests they might correspond to the formation of nanometer scale domains thus supporting the lipid raft hypothesis.

Symposium: Mitosis Studied with Biophysical Tools

1122-Symp

How Kinetochores Promote Accuracy in Mitosis: Tension, Phosphoregulation, and the Chinese 'Finger Trap' Effect

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An exquisite molecular machine, the mitotic spindle, organizes and separates chromosomes during cell division. To uncover how this machine operates we are reconstituting spindle functions and applying advanced biophysical tools for manipulating and tracking individual molecules. My talk will focus on kinetochores, the multi-protein organelles that link chromosomes to spindle microtubules, thereby driving chromosome movement. Kinetochores also perform vital regulatory activities that ensure the accuracy of mitosis. For example, a popular view is that tension selectively stabilizes proper kinetochore-microtubule attachments. Proper ('bi-oriented') attachments come under tension from opposing microtubules, and this tension is thought to cause their stabilization. Conversely, improper attachments lack tension, so they fail to become stabilized and detach quickly, giving another chance for proper attachments to form. We recently used laser trapping-based assays to show that tension stabilizes attachments between individual kinetochore particles purified from budding yeast and single microtubule tips. The tension in this case acts directly on the kinetochore-microtubule interface, causing it to adopt a more stable configuration in a manner similar to a Chinese 'finger trap' toy, or to the catch bonds that enhance cell-cell adhesion. Now we are investigating how this direct stabilization works in tandem with phosphoregulation. Aurora B kinase is known to promote mitotic accuracy through phosphorylation of kinetochore subcomplexes. Current efforts toward understanding how phosphorylation affects kinetochore function will be discussed.

1123-Symp

Visualizing the Interaction of Kinetochores Complexes with Microtubules

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Cryo-electron microscopy (Cryo-EM) has become a major tool in the structural characterization of large macromolecular assemblies, their architecture, interactions with different ligands, and the regulation of their function. I will present two different examples of how cryo-EM is being used in my lab to understand the molecular mechanisms of complex biological systems.

During division the eukaryotic cell needs to accurately segregate its genetic material between daughter cells. This process involves the interaction of the microtubule mitotic spindle with special regions on chromosomes called kinetochores. Errors, which result in misplaced chromosomes, can lead to cancer or death. We have visualized the interaction of microtubules with two kinetochore components, the yeast Dam1 and the human Ndc80 complexes, using cryo-electron microscopy and image reconstruction. Interestingly, both complexes oligomerize on the surface of the microtubule, a property that is essential for their capacity for harness the energy of microtubule depolymerization for chromosome movement.

1124-Symp

Systems Biochemistry and Structural Biology of Microtubule End Tracking

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The microtubule cytoskeleton performs essential mechano-chemical tasks in eukaryotic cells. It is crucial for the internal organisation of the cell, intracellular trafficking and for the separation of the genetic material during cell division. These complex processes require the coordinated activity of dynamic microtubules, molecular motors, their regulators, and other proteins linking microtubules to intracellular substructures. All these proteins form a dynamically interconnected microtubule cytoskeleton whose distinct biological function is intimately linked to its overall organisation and dynamic state. How this network operates as an integrative system and how its large-scale behaviour depends on the combinatorial action of its nano-scale biochemical constituents is a major open question. Biochemical reconstitutions of cytoskeletal subsystems mimicking in vivo behaviour, in combination with

quantitative fluorescence microscopy and cryo-electron microscopy, have become a powerful approach to extract the underlying rules of how the microtubule cytoskeleton acts as a dynamic system. For example, through this approach the hierarchical functioning of a regulatory protein interaction network at growing microtubule ends, formed around so-called end binding (EB) proteins, can now be understood from the atomic to the micrometer scale. In the future, the challenge will be to reconstitute even more complex systems to be able to test directly our understanding of higher-order cytoskeletal functions.

1125-Symp
The Forces that Center the Mitotic Spindle
Jonathon Howard[1], Horatiu Fantana[1], Jacques Pecreaux[2], Carlos Garzon-Coral[1], Stefanie Redemann[1], Anthony A. Hyman[1].
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Precise positioning of the mitotic spindle is important for specifying the plane of cell division and the subsequent partitioning of the cell's contents to the daughter cells. Studies on different organisms and cell types have suggested diverse centering mechanisms: astral microtubules grow out from the spindle and push against the cortex, cortical dynein motors pull on astral microtubules, and dynein-dependent organelle transport on astral microtubules leads to a reactive force on the spindle. The different mechanisms lead to different predictions for the precision of centering, how mutations effect the precision, and the magnitude of the forces associated with spindle centering. We used image processing to accurately track the position and orientation of the mitotic spindle during the first cell division in the C. elegans embryo. The high precision of centering, < 1% of cell diameter transverse to the anterior-posterior axis, increased after RNAi against gpr-1/2, genes encoding activators of the cortical force generators; this suggests that centering is not mediated by gpr-1/2-dependent cortical pulling forces. To measure the forces associated with spindle positioning, we built a magnetic tweezers apparatus so that forces could be exerted on the spindle via beads incorporated into the embryo: forces of approximately 20 pN were required to displace the spindle through 1 μm. These mechanical experiments constrain molecular models of the centering process.

Platform: Imaging & Optical Microscopy: Superresolution Imaging & Single Molecules

1126-Plat
Sub-100 nm 3D Detection Volumes by Total Internal Reflection STED Microscopy
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Stimulated Emission Depletion (STED) Microscopy (1–2) has revolutionized far-field fluorescence microscopy by breaking the classical diffraction limit: 25 nm resolution and better are routinely achieved in the focal plane.

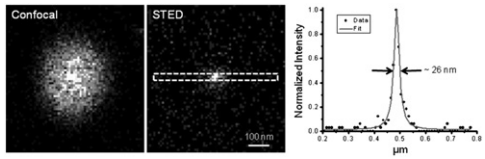

Fig. 1. 20 nm fluorescent bead imaged in confocal and STED mode. The profile across the white box demonstrates <26 nm resolution.

While comparable axial resolution values have been obtained using two opposing objectives , single-objective STED microscopy can usually not realize values on this size scale.
Here we present results obtained with a recently realized STED variant that combines total internal reflection excitation for ~70 nm axial sectioning capabilities with stimulated depletion for ~50 nm lateral super-resolution.
TIRF STED microscopy represents an attractive super-resolution alternative for live cell microscopy featuring fast scanning with sub-100 nm 3D detection volumes and reduced photo-damage through TIRF excitation.
References:
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1127-Plat
Super-Resolution dSTORM Imaging of Human Galectin-1 Interacting with Neuroblastoma Cells
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Galectins are a family of carbohydrate-binding proteins with an affinity for beta-galactosides. They share a core sequence consisting of 130 amino acids, and the beta-sandwich fold. Human Galectin-1 (hGal-1) is a well studied representative of prototype galectins, non-covalently linked homodimers with two specific carbohydrate recognition domains (CRD). It is differentially expressed by various normal and pathological tissues and is involved in intra- and extracellular processes like cell adhesion, formation of galectin-glycoprotein lattices, signal transduction and regulating immune responses, inflammation, allergies, and host-pathogen interactions. Furthermore oxidized galectins are associated with the regeneration of the central nervous system after injury.
We use direct stochastic optical reconstruction microscopy (dSTORM) to study the spatial organization of hGal-1 interacting with glycans like ganglioside GM1 presented on the membrane of human SK-N-MC neuroblastoma cells. Using the photoswitchable fluorophore ALEXA 647 as specific galectin marker, we employ fluorescence on/off switching with standard widefield microscopy and spot analysis of single molecules in order to resolve clustering, localization, and cross-linking of galectins on the cell surface with a spatial resolution of less than 50 nm.
We study spatial organization and its dependence on galectin concentration and oxidation state, as well as inhibition of the specific recognition.

1128-Plat
Direct Live-Cell Super-Resolution Imaging of Cellular DNA
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Direct stochastic optical reconstruction microscopy (dSTORM) is an imaging method that relies on the stochastic photoswitching of single fluorophores and enables resolution of structures down to tens of nanometers in biological samples. Recently, several proteins have been visualized with dSTORM in live cells by using genetically encoded tags labeled with chemical dyes, including DNA-associating proteins. However, despite its importance in cellular processes, live-cell super-resolution imaging of DNA structure itself has never been demonstrated. We present the imaging of DNA with dSTORM based on direct DNA labeling. We optimized buffer conditions to achieve the reversible photoswitching required for dSTORM in living cells and used it to resolve nuclear and mitochondrial DNA structures. Furthermore, due to the excellent preservation of the dyes, we were able to perform time-lapse super-resolution imaging. This illustrates that is possible to monitor the sub-diffraction limited organization of DNA in individual cells over time.
This protocol in combination with protein super-resolution imaging provides an advantageous tool to study processes related to DNA dynamic structural rearrangements such as those occurring during cell division or in response to cell stress.

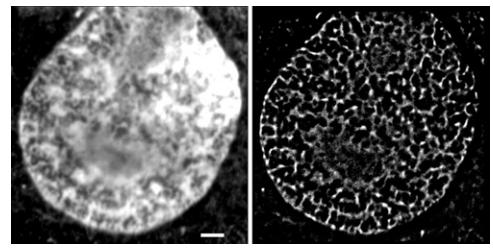
Figure: Wide-field (left) and dSTORM (left) images of cellular nucleus. Scale bar 2.5μm.

1129-Plat
Live Cell Super-Resolution Imaging of Transverse Membrane Tubules in Heart Failure
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Transverse tubules (TTs) are hollow intracellular membrane structures, coupling action potential propagation at the cell surface to intracellular